



## HCV Quantification Kit v1

### USER MANUAL

For *in vitro* Diagnostic Use



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## 1. PRODUCT DESCRIPTION

Bosphore® HCV Quantification Kit v1 detects and quantitates Hepatitis C Virus RNA in human serum and plasma, encompassing all the 6 major HCV genotypes. The linear range of quantitation is from  $1 \times 10^1$  IU/ml to at least  $1 \times 10^9$  IU/ml, and the analytic sensitivity is 25 IU/ml. A region within the 5'UTR is amplified and fluorescence detection is accomplished using the FAM filter.

An internal control has been integrated into the kit in order to check PCR inhibition. The amplification data of the internal control is detected with the Cy5 filter. The internal control can be added either during RNA extraction or PCR step.

## 2. CONTENT

Bosphore® HCV RNA Quantification Kit v1 is composed of Real-Time RT PCR reagents and quantitation serum standards which have been calibrated against WHO International Standard (NIBSC Code 06/100):

Component	REAGENT	100 Tests	50 Tests	25 Tests
1	dH <sub>2</sub> O	(1000 µl)	(1000 µl)	(500 µl)
2	PCR Mix	(2240 µl)	(1120 µl)	(560 µl)
3	RT Mix	(45 µl)	(23 µl)	(12 µl)
4	Detection Mix1	(255 µl)	(127 µl)	(63 µl)
5	Detection Mix2	(134 µl)	(67 µl)	(33 µl)
6	Internal Control	(560 µl)	(280 µl)	(140 µl)
7	Positive Control 1	(70 µl)	(35 µl)	(17 µl)
8	Standard 1 ( $1 \times 10^6$ ) IU/ml	(880 µl)	(880 µl)	(440 µl)
9	Standard 2 ( $1 \times 10^5$ ) IU/ml	(880 µl)	(880 µl)	(440 µl)
10	Standard 3 ( $1 \times 10^4$ ) IU/ml	(880 µl)	(880 µl)	(440 µl)
11	Standard 4 ( $2 \times 10^3$ ) IU/ml	(880 µl)	(880 µl)	(440 µl)

## 3. STORAGE

Bosphore® HCV Quantification Kit v1 PCR reagents should be stored at -20°C. Repeated thawing and freezing (>3x) should be avoided since it may reduce sensitivity. If the components are to be used in small amounts, they should be frozen in aliquots.

While preparing the PCR; the components should not be exposed to room temperature for more than 10 min. and the detection mix components should not be exposed to light more than 1-2 min. We recommend preparing the PCR on a cooling block and keeping the detection mixes within a closed container.

The components maintain their stability until the expiry dates on the labels, if they are stored at advised conditions.

## 4. REQUIRED MATERIALS AND DEVICES

- Montania® 483 Real-Time PCR Instrument (Anatolia Geneworks), or another Real-Time PCR system with FAM and Cy5 filters (iCycler, iQ5, CFX-BioRad, LightCycler 1.5, 2.0, 480-Roche, 7500 Real-Time PCR System-ABI, Stratagene Mx3005P, Mx3000P-Agilent, LineGeneK, LineGene 9600-Bioer, Rotorgene 2000, 3000, 6000, Q-Qiagen)
- 0.2 ml Thin-Wall PCR tubes or strips
- Magnesia® 16 Nucleic Acid Extraction System / Magnesia® Viral Nucleic Acid Extraction Kit (Anatolia Geneworks), or other high quality viral RNA extraction kits and systems
- Deep freezer (-20°C)
- Desktop centrifuge with rotor for 2 ml. microcentrifuge tubes
- Calibrated adjustable micropipettes

- DNase, RNase, pyrogen free micropipette tips with filters
- DNase, RNase, pyrogen free 1.5 or 2 ml. microcentrifuge tubes
- Disposable laboratory gloves

## 5. IMPORTANT NOTES AND SAFETY INSTRUCTIONS

### Important!:

- The product should be delivered on dry ice. Check for presence of dry ice upon arrival.
- Check for the expiry dates on the box and tube labels, upon arrival. Do not use expired products or components.
- Calibrated or verified micropipettes, DNase, RNase, pyrogen free micropipette tips with filters, and DNase, RNase, pyrogen free microcentrifuge tubes should be used.
- Before starting a test procedure, all components should be thoroughly thawed. After thawing, all components should be centrifuged briefly (spin-down for 3-5 seconds), and mixed well to ensure homogeneity prior to use.
- The kit components should be kept on ice or a cooling block until the reaction is prepared, and they should be quickly returned to -20°C.
- PCR and nucleic acid isolation must be performed in different compartments. Samples should be stored separately to avoid contact with the kit components.
- Pathogen information should be reviewed to be aware of the health related risks.
- Serum/plasma samples (including the standards) should be handled with extreme caution, suitable class microbiological safety cabinet should be used: Physical contact with pathogens should be avoided by; wearing lab coats and gloves, no allowance for eating or drinking within the workspace, prevention of unauthorized individuals' access to the working area.
- All the pathogenic wastes produced during the nucleic acid isolation step; including the serum samples and material contacted with them, should be discarded into medical waste and disposed safely.

## 6. PRODUCT USE LIMITATIONS

- All the components may exclusively be used for in vitro diagnostics.
- This product should be used in accordance with this user manual, by personnel specially trained to perform in vitro diagnostic procedures.

## 7. PATHOGEN

### Causative Agents

The hepatitis C virus is a hepacivirus of the Flaviviridae family of viruses that causes Hepatitis C in humans. It is a small, enveloped, 9.6kb single-stranded RNA virus that is classified into six main genotypes (1-6) with more than one hundred different subtypes. [1]

### Epidemiology

It is estimated that HCV has a worldwide prevalence of 3% affecting around 180 million people with between 3 to 4 million new infections each year. The vast majority of infected people (70-90%) develop chronic infection.

Though chronic infection may be asymptomatic, it is a leading cause of chronic liver diseases, including cirrhosis in between 20 to 50% of patients. Treatment may be effective in 10-50% of patients depending on the applied therapy. [2]

#### **Modes of Transmission:**

Hepatitis C is believed to be spread through contact with infected blood. However, unlike many other blood borne viruses, HCV may be transmitted even through indirect sources like a used razor, making HCV more transmissible than other blood borne viruses –including HIV. Common routes of transmission include transfusion of blood products, intravenous and percutaneous drug and needle use, work accidents among healthcare workers and any other blood to blood contacts, such as sexual practices and from mother to newborn (maternal-infant transmission). Statistical studies have revealed no risk factors for HCV transmission in the activities of daily living (sneezing, coughing, hugging, etc.). [2], [3]

### **8. METHOD**

Bosphore® HCV Quantification Kit v1 is based on the Real Time RT PCR method. HCV genetic material is amplified by reverse transcription technique since it is composed of RNA. RT-PCR, which is also referred as RNA PCR, is a two-step reaction. First, complementary DNA is synthesized from RNA by reverse transcription and then complementary DNA is amplified by standard PCR. The primer binds to the target RNA region in RT-PCR and RNA-DNA double strand is synthesized by reverse transcriptase enzyme using the RNA template for complementary DNA. Afterwards, standard PCR continues.

Polymerase chain reaction is a technique that is used for amplification of a DNA region. The reaction occurs by the repeating cycles of heating and cooling. The main components of PCR are primers, dNTPs, Taq polymerase enzyme, buffer solution and template. As a brief explanation, primers are small synthetic DNA those anneal to the specific regions of the template in order to start the synthesis. dNTPs are the building blocks of the amplified products. Taq polymerase amplifies the DNA template. Buffer solution provides the pH adjustment required for the reaction and template, as referred, is the target region for synthesis. In addition to these components, in RT PCR reverse transcriptase is added to the reaction and cDNA synthesis from the RNA template is acquired.

In Real Time PCR technique, in contrast to conventional PCR, PCR product can be monitored during the reaction. Therefore Real-Time PCR obviates the need for further analysis methods like gel electrophoresis, whereby minimizing the risk of contamination. Dual labeled probes employed in the reaction in addition to the conventional PCR reagents, enable detection of the amplified target with increased sensitivity. I

The assay utilizes the 5' exonuclease activity of Taq Polymerase to cleave a dual-labeled fluorescent hybridization probe during the extension phase of PCR.

The probe is labeled at the 5' end with a fluorescent 'reporter' molecule, and at the 3'end with another fluorescent molecule that acts as a 'quencher' for the 'reporter'. When the two fluorophores are in close proximity, and the reporter is excited by light, no reporter fluorescence can be detected. During the elongation step of PCR, Taq Polymerase encounters and cleaves the probe bound to the template. As the reporter is freed from the suppressing effect of the quencher, fluorescence signal can be detected.

The fluorescence generated by the reporter increases as the PCR product is accumulated; the point at which the signal rises above background level and becomes distinguishable, is called the threshold cycle ( $C_T$ ). There is a linear relationship between the log of the starting amount of a template and its threshold cycle, thus starting amount of

unknown templates can be determined using standard curves constructed using  $C_T$  values of the known starting amounts of target templates.

Bosphore HCV Quantification Kit v1 employs multiplex PCR, and an internal control is incorporated into the system in order to control the isolation procedure and to check for possible PCR inhibition. HCV RNA (cDNA) and an internal control are co-amplified in a single reaction, using sequence-specific primers. The fluorescent signal generated by the HCV amplification is detected by a probe labeled at the 3' end with FAM, through the FAM channel. The fluorescent signal generated by the internal control amplification, is detected by a second probe (labeled at the 5' end with a different reporter molecule, Cy5) through the Cy5 channel.

## **9. PROCEDURE**

### **9.1. Sample Preparation, Storage and Transport**

To isolate serum from the clinical specimen, the blood sample should be collected into sterile vacutainers without any anticoagulant. For venipuncture, only sterile material should be used.

The serum should be separated from blood within 6 hours after blood collection. To separate the serum, the blood container should be centrifuged at 800-1600 x g for 20 minutes. The separated serum should be transferred to polypropylene tubes and stored at -20°C or lower, until use.

The samples should be transported in containers with capacity to resist pressure. Transportation should be done according to local and national regulations for pathogen material transport.

### **9.2. Interfering Substances**

The following factors may have possible influences on PCR:

- Hemolytic samples,
- Samples of heparinized patients,
- Samples of patients with elevated levels of bile salts, bilirubin or lipids

### **9.3. RNA Isolation**

We recommend that the Magnesia® 16 Nucleic Acid Extraction System / Magnesia® Viral Nucleic Acid Extraction Kit (Anatolia Geneworks) isolation system is used with Bosphore® HCV Quantification Kit v1. The RNA isolation should be performed according to the manufacturers' instructions. The starting volume is 400 µl, the elution volume is 60 µl and the amount of internal control that should be used during isolation for each system is 5 µl. The external quantitation standards are provided as serum, so that they undergo the same steps as the patient samples, starting from RNA isolation.

### **9.4. Kit Components**

#### **9.4.1. PCR Mix**

PCR mix contains; HotStarTaq DNA Polymerase, Probe RT-PCR Buffer, and ROX passive reference dye.

HotStarTaq DNA Polymerase: HotStarTaq DNA Polymerase is a modified form of Taq DNA Polymerase, and is provided in an inactive state and has no enzymatic activity at ambient temperature. The enzyme remains completely inactive during the reverse-transcription reaction and does not interfere with it. This prevents formation of misprimed RT-PCR products and primer-dimers during reaction setup, reverse transcription, and the first denaturation step. The enzyme is activated after the reverse-transcription step by a 15-minute, 95°C incubation step. The hot start also inactivates the reverse-transcription enzymes, ensuring temporal separation of reverse transcription and PCR, and allowing both steps to be performed sequentially in a single tube.

Probe RT-PCR Buffer: It is a unique OneStep RT-PCR buffer system, and has been specifically adapted for realtime RT-PCR using sequence-specific probes. The buffer contains a balanced combination of KCl and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>.

ROX passive reference dye: For certain real-time cyclers, the presence of ROX passive reference dye in real-time PCR compensates for non-PCR-related variations in fluorescence detection. Fluorescence from ROX dye does not change during the course of real-time PCR, but provides a stable baseline to which PCR-related fluorescent signals are normalized. Thus, ROX dye compensates for differences in fluorescence detection between wells due to slight variations in reaction volume or to differences in well position. The use of ROX dye is necessary for all instruments from Applied Biosystems and is optional for instruments from Stratagene (e.g., Mx3000P®, Mx3005P®, and Mx4000®). Montania 483, Rotor-Gene cyclers and instruments from Bio-Rad/MJ Research, Cepheid, Eppendorf, and Roche do not require ROX dye. The presence of ROX dye in the master mix does not interfere with real-time PCR on any instrument, since the dye is not involved in the reaction and has an emission spectrum completely different from fluorescent dyes commonly used for probes.

#### **9.4.2. RT mix**

RT Mix contains a unique Omniscript and Sensiscript blend. Both enzymes exhibit a high affinity for RNA, facilitating transcription through secondary structures that may inhibit other reverse transcriptases. Omniscript is designed for reverse transcription of RNA amounts greater than 50 ng, and Sensiscript is optimized for use with very small amounts of RNA (<50 ng). This enzyme combination provides highly efficient and sensitive reverse transcription over a wide range of RNA template amounts.

#### **9.4.3. Detection Mix 1**

Detection Mix 1 contains HCV-specific forward and reverse primers and a dual-labeled probe.

#### **9.4.4. Detection Mix 2**

Detection Mix 2 contains internal control-specific forward and reverse primers and a dual-labeled probe.

#### **9.4.5. Internal Control**

An internal control is included in the kit to control RNA isolation and PCR inhibition. The internal control is a synthetic DNA molecule derived from human genome. It is added into the serum, proteinase K and carrier RNA mixture during DNA isolation, to control the isolation efficiency and PCR inhibition. The amount of IC that should be added during isolation is 5 µl per serum sample. Alternatively, the internal control can be added directly into the PCR master mix to control the PCR inhibition exclusively. For this purpose, 0.4 µl of internal control should be added for each reaction into the master mix. Lack of internal control amplification in the FAM negative samples, may indicate a problem in isolation or PCR inhibition. In this case, isolation and PCR should be repeated. In samples that contain a high viral load, internal control can be suppressed and no increase of the signal is detected. Please use the table below for the interpretation of internal control data:

HCV(FAM)	Internal Control (Cy5)	Interpretation
+	+	Sample positive
-	+	Sample negative
+	-	Sample positive
-	-	Repeat the test!

#### **9.4.6. Positive Control**

The positive control contains HCV RNA. It can be included in the PCR to test the efficiency of the PCR exclusively. The threshold cycle for the positive control is given in the acceptance criteria table (Section 10. Analysis). Threshold cycles higher than the acceptance criteria may indicate an efficiency loss in the reaction.



#### 9.4.7. Quantitation Standards

The quantitation serum standards are calibrated by WHO International Standard (NIBSC Code 06/100).

#### 9.5. Preparing the RT-PCR

All four external quantitation standards should be added into the PCR reaction together with the samples and the negative control (PCR-grade water). Make sure that all the kit components are thawed before use. Refer to the table below for preparing the PCR. It is for only one reaction, multiply these values with the sample number to find the values required for the master mix. While preparing master mixes for more than 5 samples, an extra 10% should be added to the total sample number.

When the Internal Control is added in the extraction step:

<b>PCR Mix</b>	20 µl
<b>RT-mix</b>	0.4 µl
<b>Detection Mix 1</b>	2.28 µl
<b>Detection Mix 2</b>	1.2 µl
<b>dH2O</b>	0.12 µl
<b>Sample RNA (Standard, Negative/Positive Control)</b>	16 µl
<b>Total Volume</b>	40 µl

When the Internal Control is added in the PCR step:

<b>PCR Mix</b>	20 µl
<b>RT-mix</b>	0.4 µl
<b>Detection Mix 1</b>	2.28 µl
<b>Detection Mix 2</b>	1.2 µl
<b>Internal Control</b>	0.4 µl
<b>Sample RNA (Standard, Negative/Positive Control)</b>	16 µl
<b>Total Volume</b>	40.28 µl

Pipette 24 µl of the master mix into the PCR tubes or strips, and add 16 µl of RNA (sample/ standard/positive or negative control). Close the tube cap. Make sure that the solution in each tube is at the bottom of the tube. Centrifuge if necessary.

#### 9.6. Programming the Montania® 483 Real-Time PCR Instrument

The thermal protocol for Bosphore® HCV Quantification Kit v1 is composed of two-steps; firstly a reverse transcription step and secondly Real Time PCR steps; an initial denaturation for activation the HotStarTaq DNA Polymerase, a two-step amplification cycle and a terminal hold. The real-time data is collected at the second step of the amplification cycle.

Reverse Transcription	50°C	30:00 min.	} 50 cycles
Initial denaturation	95°C	14:30 min.	
Denaturation	97°C	00:30 min.	
Annealing (Data Collection)	55°C	01:20 min.	
Synthesis	72°C	0:15 min.	
Hold	22°C	05:00	

Before starting a Real-Time PCR reaction using the Bosphore® Kits, the following steps should be completed:

- Choose the filter pairs to be used (FAM and Cy5),
- Identify unknown samples, standards, positive and negative controls, assign quantitative values to the standards,
- Select the correct thermal protocol.

These steps are described below:

From the main menu of the Montania® 483 Real-Time PCR Instrument, "File" and then "New" is selected. "Create a new Experiment" is selected. In the "Select Channel" window channels 1 (FAM) and 3 (Cy5) are selected (Fig. 1). Standards, samples and negative controls are identified in the "Module Edit" menu (Fig. 2). Standards should only be defined for the FAM channel and their concentration/viral load should be entered. To select the thermal protocol "Gene Amplification" menu is used. The "Open" button in the "Experiment Program" is clicked and the appropriate thermal protocol is selected. (Fig. 3a). The thermal cycles of the selected protocol is displayed. The experiment starts by clicking the "Start" button (Fig. 3b).

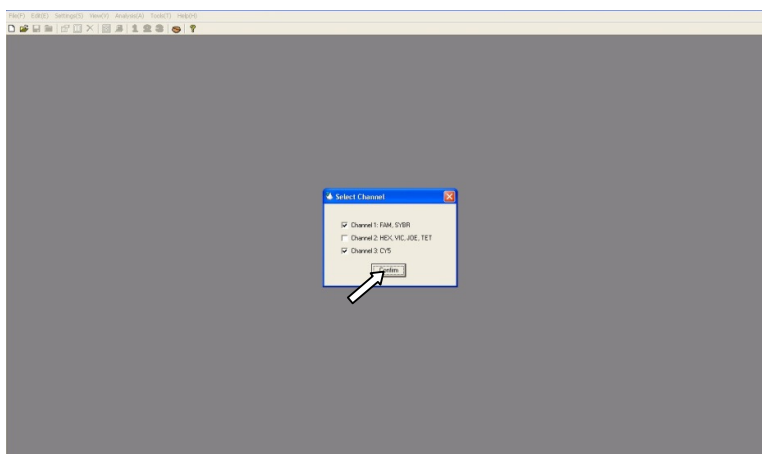


Fig. 1: Filter Selection in Montania® 483

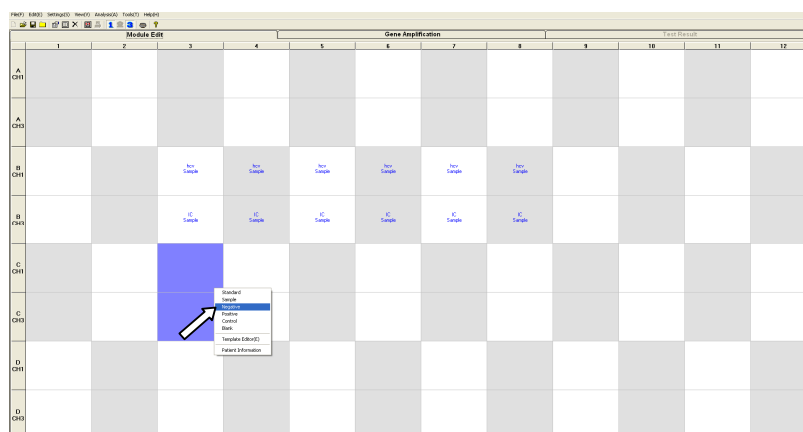


Fig. 2: Sample Location and Identification

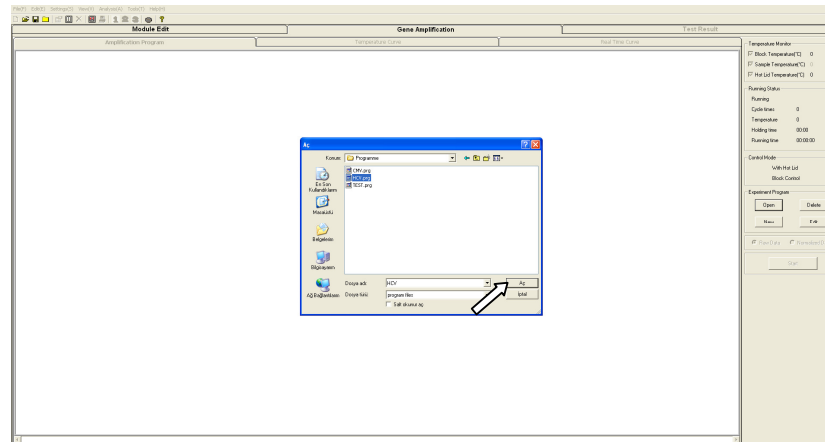


Fig. 3a: Selecting the Thermal Protocol

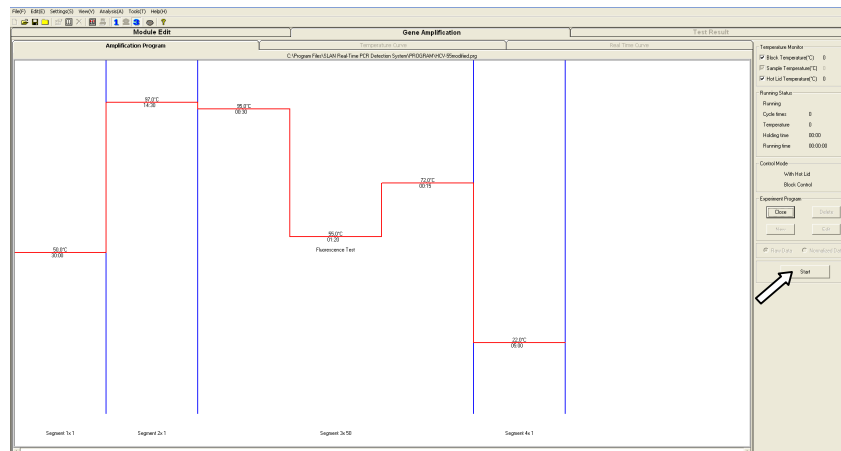


Fig. 3b: Starting the Experiment

## 10. ANALYSIS

By the end of the thermal protocol, the Montania® 483 Real-Time PCR Instrument software automatically calculates the baseline cycles and the threshold.

Example of an amplification curve is given in Fig. 4.

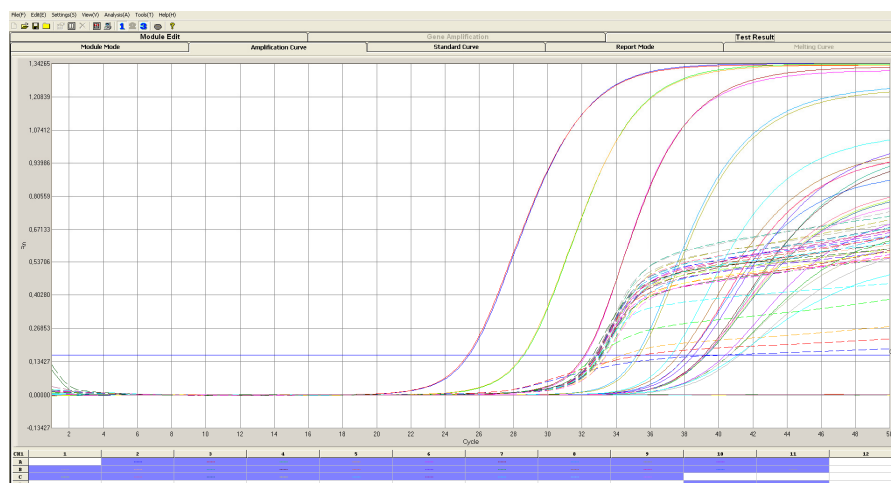


Fig. 4: Amplification Curve of a Bosphore® HCV v1 test

The standard curve is plotted using the data obtained from the defined standards, with the axes Ct-Threshold Cycle and Log Starting Quantity. Example of a standard curve is given in Fig. 5

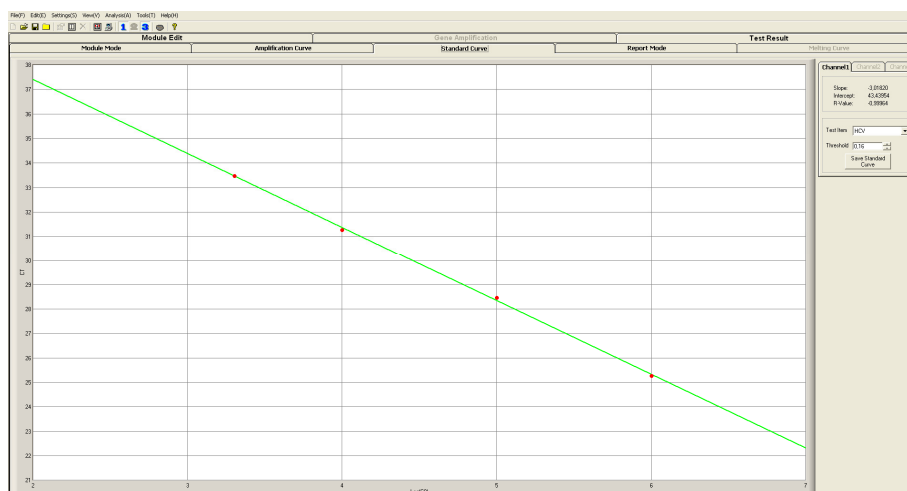


Fig.5: Standard Curve of a Bosphore® HCV v1 test

Analysis of the results should be performed by trained personnel who have received the required training for analysing Real-Time PCR data. We recommend that the test results must be evaluated by an expert clinician, taking the patient's clinical findings and the results of other tests into consideration.

All analysis is done automatically in routine use. However, when the trained personnel, who have received the required training from manufacturer, consider it as necessary, the system allows pulling down the threshold as much as possible in order to detect low positive samples. In this case, attention should be paid to keep the threshold line above the background and to keep the correlation coefficient at the maximum possible value (and within its acceptance criteria.)

The table below displays the acceptance criteria for Bosphore® HCV v.1.

Component/Parameter	Cycle Threshold (C <sub>T</sub> )
Standard 1	25±2.5
Standard 2	28±2
Standard 3	31±2
Standard 4	33±2
Positive Control	33±4
Correlation Coefficient	>0.950
PCR Efficiency*	>%60

\* PCR efficiency is calculated by the following formula:  $10^{(-1/\text{slope})} - 1 \times 100$

Test results should not be reported unless the assay results meet the criteria stated above. Please contact the manufacturer if an impairment in the product's performance is observed (See the last page for contact information).

The quantitative results of the test are displayed on the "Report Mode" screen. A spread sheet containing the calculated starting quantities of the unknown samples in each tube is shown. The samples that cross the threshold in Channel 1 (FAM) are displayed with a calculated starting quantity, samples that do not cut the threshold are displayed as "No Ct". These samples are regarded as negative or having a viral load below the detection limit of the

assay. For these undetectable samples, the Cy5 data of the internal control should also be checked to avoid false negative results (Fig. 6).

Sample ID	Channel	Type	Test Item	Property	CT	Test Result	Label	Name	Sex	Age	Case ID	Bed ID	Inpatient ID	Outpatient ID	Sample Type	Sample Date	Diagnosis	Doctor	Detect Date	Office	Experiment	Assessor	Result	Melting Curve
A10	1	Sample	HCV		40.26	1.414E+01																		
	3	Sample	IC		33.07	No Result																		
B3	1	Sample	HCV		39.23	3.000E+01																		
	3	Sample	IC		32.88	No Result																		
B4	1	Sample	HCV		39.18	3.862E+01																		
	3	Sample	IC		33.11	No Result																		
B5	1	Sample	HCV		No Ct	0.000E+00																		
	3	Sample	IC		32.72	No Result																		
B6	1	Sample	HCV		38.49	5.798E+01																		
	3	Sample	IC		32.83	No Result																		
B7	1	Sample	HCV		40.59	1.188E+01																		
	3	Sample	IC		33.09	No Result																		
B8	1	Sample	HCV		37.62	1.122E+02																		
	3	Sample	IC		32.82	No Result																		
C1	1	Sample	HCV		39.26	3.266E+01																		
	3	Sample	IC		33.12	No Result																		
C2	1	Sample	HCV		39.16	3.400E+01																		
	3	Sample	IC		32.80	No Result																		
C3	1	Sample	HCV		39.26	3.266E+01																		
	3	Sample	IC		32.78	No Result																		
C4	1	Sample	HCV		No Ct	0.000E+00																		
	3	Sample	IC		32.35	No Result																		
C5	1	Sample	HCV		37.19	1.953E+02																		
	3	Sample	IC		33.16	No Result																		
C6	1	Sample	HCV		No Ct	0.000E+00																		
	3	Sample	IC		33.13	No Result																		
C7	1	Sample	HCV		41.89	8.123E+00																		
	3	Sample	IC		33.04	No Result																		
C8	1	Sample	HCV		No Ct	0.000E+00																		
	3	Sample	IC		33.00	No Result																		
C9	1	Sample	HCV		39.17	3.400E+01																		
	3	Sample	IC		33.40	No Result																		
D10	1	Negative	HCV		No Ct	0.000E+00																		
	3	Negative	IC		32.97	No Result																		
D11	1	Negative	HCV		No Ct	0.000E+00																		
	3	Negative	IC		32.83	No Result																		

Fig. 6: A Report Mode Screen Showing the Results

The following table shows the possible results and their interpretation:

Signal detected in FAM filter pair	The sample contains HCV RNA, the result is positive	No need to check the internal control since the sample is positive (high positive samples may suppress the signal from the internal control)
No signal in FAM, signal in Cy5	The HCV RNA in the sample is not detectable	Signal from Cy5 filter pair rules out the possibility of PCR inhibition
No signal in FAM and Cy5	The diagnosis is inconclusive	No signal in Cy5 points out to PCR inhibition or to a problem in RNA isolation (See 11.Troubleshooting)

## 11. TROUBLESHOOTING

Please contact the manufacturer in case of a problem during a run.

Late or no signal from the FAM filter

Wrong thermal protocol is chosen	Make sure that the correct thermal protocol is chosen.
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Late or weak signal from the standards

Deterioration of the standards or the core kit components	Don't use expired standards or kit components. Follow the instructions for the storage of kit components (Section 3. Storage).
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No signal from the internal control

Deterioration of the internal control or detection mix 2	Follow the instructions for the storage of kit components (See Section 3. Storage).
PCR inhibition	Make sure that you use the recommended RNA isolation method (See 9.3 RNA isolation).
RNA lost during isolation	Make sure that you use the recommended RNA isolation method (See 9.3 RNA isolation).

Signal from FAM Filter in the Negative Control

Contamination	Use filter-tips. Repeat PCR with new kit components.
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The Threshold is Above Low Signals

The threshold should	Using the mouse pull the threshold down until it cuts the low
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be manually adjusted	signals. Avoid the background and the signal from negative control.
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## 12. SPECIFICATIONS

### 12.1. Sensitivity

Analytical sensitivity may be expressed as the limit of detection: i.e. the smallest amount of the target marker that can be precisely detected. The detection limit of an individual analytical procedure is the lowest amount of nucleic acid in a sample which can be detected but not necessarily quantitated as an exact value. The analytical sensitivity or detection limit for NAT assays is expressed by the 95% positive cut-off value.

The analytical detection limit for Bosphore® HCV v1 was found to be  $2.5 \times 10^1$  IU/ml ( $p=0.05$ ). The sensitivity was determined using serial dilutions of RNA calibrated with the WHO International Standard for HCV RNA NAT assays, (NIBSC Code 06/100). The dilutions were tested in different runs in replicates. The results were analyzed by probit method.

#### 12.1.1. Genotype Detection and Quantitation Efficiency

Efficiency of detecting and quantitating different genotypes were ensured both by sequence comparison analysis and a Real-Time PCR assay using Worldwide HCV Performance Panel WWHV302(M) (Seracare). The following genotypes were tested and found positive:

Panel Member	Genotype	HCV(FAM)
1	1b	+
2	1a	+
3	1b	+
4	2a/2c	+
6	3b	+
8	3a	+
10	4	+
11	4	+
12	5a	+
14	6a	+

### 12.2. Linear Range

The linear range of Bosphore® HCV Quantification Kit v1 was determined to be from  $1 \times 10^1$  IU/ml to at least  $1 \times 10^9$  IU/ml.

In order to assess the linear range, a dilution series which has been calibrated against the WHO International Standard for HCV RNA NAT assays, (NIBSC Code 06/100) was analyzed by testing each dilution in 2 replicates (Fig. 7a and 7b). The standard curve correlation coefficient was found to be 0.99802.

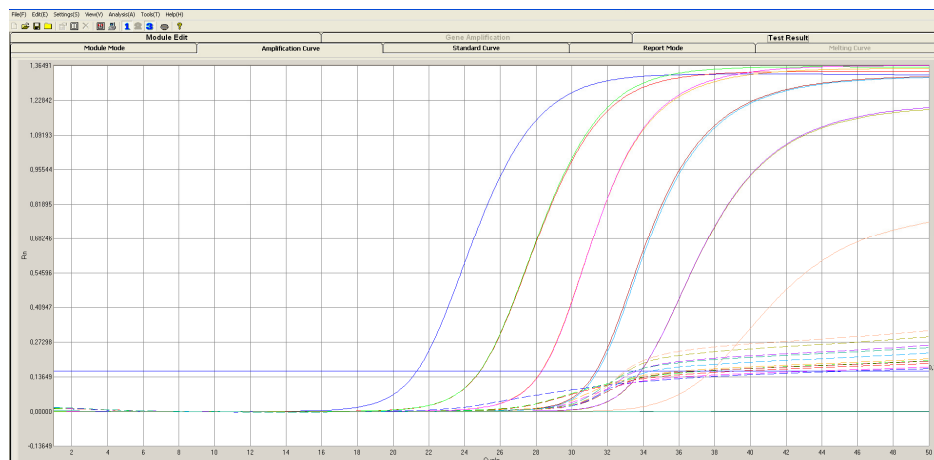


Fig. 7a: Linear Range Amplification Curve

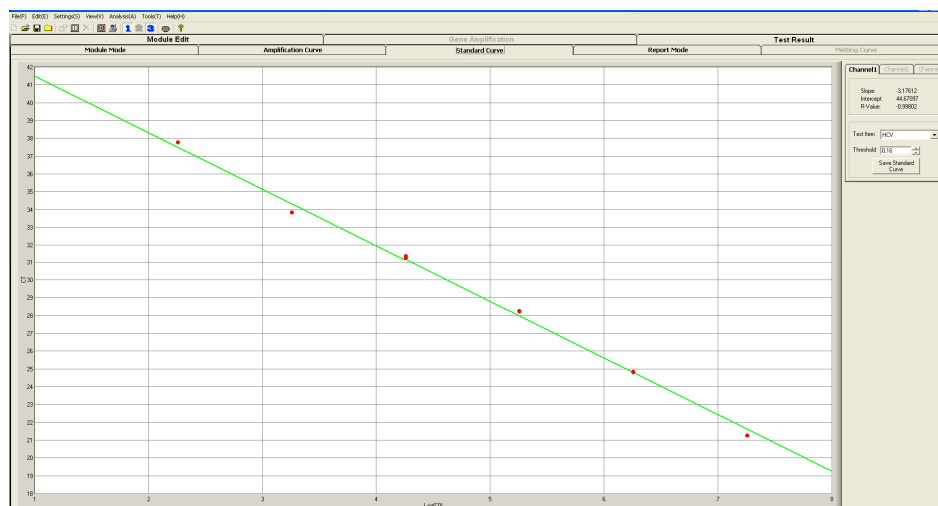


Fig. 7b: Linear Range Standard Curve

### 12.3. Cross-Reactivity

To eliminate potential cross-reactivity, both assay design evidence and experimental studies were employed. Primer and probe sequences were checked for possible homology to other known pathogen sequences by sequence comparison analysis using database alignment. Samples of HIV, HDV, HBV with known high positivity were tested, and found negative.

### 12.4. Reproducibility and Precision

Reproducibility data (on  $C_T$  value basis) were obtained by the analysis of one of the quantitation standards of the Bosphore® HCV Quantification Kit v1. Test was performed in at least 4 replicates by 3 different operators, on multiple days, using 3 different lots. The resulting data is given in Table 1 and Table2:

Table 1: Reproducibility Data.

HCV (10 <sup>4</sup> IU/ml)	Standard deviation	Variance	Coefficient of variation [%]
<b>Intra-assay Variability N=4</b>	0.03	0.001	0.11
<b>Inter-lot Variability N=3</b>	0.29	0.08	0.92
<b>Inter-operator Variability N=3</b>	0.26	0.08	0.92
<b>Total Inter-assay Variability N=5</b>	0.25	0.06	0.81

Table 2: Precision Data.

HCV (10 <sup>4</sup> IU/ml)	Measured Quantity (MQ) IU / ml	Standard Deviation (MQ)	Coefficient of variation [%] (MQ)	Threshold Cycle (Ct)	Standard Deviation (Ct)
<b>Intra-assay Variability N=4</b>	10852,5	267,87	2,46	31,24	0,03
<b>Inter-lot Variability N=3</b>	11072,17	1074,97	9,70	31,41	0,29
<b>Inter- operator Variability N=3</b>	9290,58	1354,50	14,57	31,53	0,26
<b>Total Inter- assay Variability N=5</b>	10047,15	1596,95	15,89	31,52	0,25

### 12.5. Diagnostic Evaluation

The diagnostic evaluation was performed by testing 100 HCV negative and 5 HCV positive serum samples which have been previously analyzed using Roche Diagnostics Elecsys 2010. All of the negative samples were found negative, and all of the positive samples were found positive with Bosphore® HCV Quantification Kit v1. 17 HCV positive and 2 negative serum samples, which have been previously analyzed using Roche COBAS Amplicor HCV RNA Monitor v2.0, Bayer Versant HCV RNA v3.0, Abbott HCV RNA m2000 and Roche HCV RNA Taqman, were tested with Bosphore® HCVQuantification Kit v1. All the positive samples were found to be positive, and all the negative samples were found to be negative.

### 12.6. Calibration Against WHO Standard

Quantitation Standards were calibrated against the WHO International Standard for HBV DNA NAT assays (NIBSC Code 06/100). 1 IU was found to be equal to  $3 \pm 0,2$  copies/ml .

## 13. REFERENCES

1. By K. E. Nelson, C. Williams, and N. Graham., Infectious Disease Epidemiology: Theory and Practice, July 15, 2000 , p :923-926



2. Theodore Sy and M. Mazen Jamal, Epidemiology of Hepatitis C Virus (HCV) Infection, Int J Med Sci. 2006; 3(2), p:41–46.
3. Anonymous, *Hepatitis C Fact Sheet No. 164*. 2000, World Health Organization.

#### 14. SYMBOLS



Use by



Lot/Batch



Catalog number



Temperature limitation



Caution, consult accompanying documents



Manufacturer



In Vitro Diagnostic Medical Device

#### 15. CONTACT INFORMATION



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